

AMENDMENTS

IN THE CLAIMS:

Kindly delete claim 42,

replace the indicated claims with:

29. (Amended) A method of labeling a nucleic acid molecule, comprising the steps of:

- a. hybridizing a first nucleic acid to a second nucleic acid, wherein the first nucleic acid comprises, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - i. the Substrate Hybridization Domain consists of a sequence of less than 10 nucleotides; and
 - ii. the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;

and the second nucleic acid comprises, from 3' to 5': a Template Hybridization Domain and a Target Binding Domain, wherein:

- i. the Template Hybridization Domain consists of a sequence of less than 10 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
- ii. the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain;

and:

- b. extending the second nucleic acid with a DNA polymerase in the presence of a labeled nucleotide to create an oligonucleotide having from 5' to 3' an unlabeled Target Binding Domain, a Template Hybridization Domain, and a labeled Signal Domain having a sequence which shows complementarity toward and is hybridizable to the Signal Template Domain.

34. (Amended) The method of claim 29, wherein the Substrate Hybridization Domain consists of a sequence of about 5 to less than 10 nucleotides.

51. (Amended) A kit for labeling a nucleic acid molecule, comprising a reaction mixture and a DNA polymerase, wherein the reaction mixture comprises:

- a. a first nucleic acid comprising, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - i. the Substrate Hybridization Domain consists of a sequence of less than 10 nucleotides; and
 - ii. the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;and
- b. a second nucleic acid comprising, from 3' to 5': a Template Hybridization Domain and a Target Binding Domain, wherein:
 - i. the Template Hybridization Domain consists of a sequence of less than 10 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
 - ii. the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain;
- c. wherein the hybridization domains of the first and second nucleic acids hybridize to each other under conditions in which an enzyme can extend the second nucleic acid by adding a sequence complementary to the Signal Template Domain.

and add the following new claim:

57. (new) The method of claim 29, wherein the first nucleic acid has a hairpin loop 5' to the Signal Template Domain.

Status of the Claims

Following entry of the amendments and new claim 57, claims 29-41 and 43-57 will be pending in the application.

Summary of the Amendments

Claim 29 has been amended to clarify that the Substrate and Template Hybridization Domains "consist of sequences of less than 10 nucleotides." Claim 34 has been amended to clarify that the Substrate Hybridization Domain also "consists of" sequences of "less than" 10 nucleotides. Support for these amendments can be found in the specification at page 19, line 6. Claim 29 has also been amended to clarify that the addition of labeled nucleotides by DNA polymerase creates an oligonucleotide having from 5' to 3' an unlabeled Target Binding Domain, a Template Hybridization Domain, and a labeled Signal Domain. Support for this amendment can be found in the specification in Fig. 1 at "C)" where this configuration is shown diagrammatically. Claim 51 has been amended to clarify that the first and second nucleic acids hybridize to each other under conditions in which an enzyme can extend the second nucleic acid. Support for this amendment can be found in the specification in Fig. 1 steps B) and C) which shows this amendment diagrammatically. Claim 57 has been added to specifically indicate that a hairpin loop can be disposed to the 5'-side of the Signal Template Domain in the first nucleic acid. Support for this amendment resides in the fact that this is a common feature of nucleic acid polymers for example SEQ ID NO 18 has TGA at residue positions 3, 4, and 5 which could pair with ACT residues at positions 19, 20, 21 to form a hairpin loop on the 5'-side of the Signal Template Domain. The Applicant requests that the amendment and new claim 57 be accepted and considered along with the remarks below.

Summary of the Invention

The invention is for a general method of labeling the 3' end of oligonucleotides with a defined number of detectable markers. The method produces a more uniform labeled oligonucleotide product and several-fold higher levels of marker incorporation than has previously been available.

The method involves annealing two oligonucleotides together through short

hybridization domains that consist of less than 10 nucleotides. The oligonucleotides include a hybridization probe (termed a "Substrate Nucleic Acid" in the application) and a template (termed a "Template Nucleic Acid"). The template defines which nucleotides to add to the hybridization probe and the number of additions. The hybridization probe contains a short sequence of less than 10 nucleotides, such as GGCGGG, added toward its 3'-end (termed a "Template Hybridization Domain") to facilitate hybridization with the template through the template's hybridization domain (termed a "Substrate Hybridization Domain"). The region of the template oligonucleotide that serves as a template for the addition of nucleotides containing the detectable marker (i.e. α - ^{32}P phosphate) to the 3'-end of the hybridization probe is usually a sequence of T nucleotides. This region is designated as the "Signal Template Domain." Following the annealing step, nucleotides containing detectable labels or markers are incorporated onto the 3' end of the hybridization probe using a DNA polymerase, such as the Klenow fragment. The terminology and method are diagramed below.



Labeling Reaction, add DNA polymerase and α - ^{32}P -ATP

